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Patent Agent

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RE: U.S. Patent Application No. 10/714,449
Certified Copy of the English Translation of the Priority Document (Argentina Application No. P010102313

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HOJA TECNICA

(19) I.N.P.I.
REPUBLICA ARGENTINA

(10) PUBLICACION N°: AR

(21) SOLICITUD N°:

(51) INT. CL.: P 0101023 13

(12) X PATENTE DE INVENCION MODELO DE UTILIDAD

(22) FECHA PRESENTACION:	(71) SOLICITANTE(S): BIO SIDUS S.A. - Constitución 4234 – Ciudad de Buenos Aires –AR- Fundación Universitaria Dr. René G. Favaloro. Solís 453 – Ciudad de Buenos Aires –AR-
(30) DATOS PRIORIDAD:	(72) INVENTOR(ES):
(41) FECHA PUBLICACION SOLICITUD: BOLETIN N°:	(74) AGENTE: 611
(61) ADICIONAL A:	(83) DEPOS. MICROORGANISMOS:
(62) DIVISIONAL DE:	

(54) TITULO DE LA INVENCION: METODO PARA INDUCIR LA PROLIFERACION NEOVASCULAR Y REGENERACION TISULAR.

(57) La presente invención se refiere a un método para inducir la proliferación neovascular y regeneración tisular en mamíferos. El método reivindicado se caracteriza por administrar a un tejido una secuencia de nucleótidos codificante para el sitio activo del factor de crecimiento del endotelio vascular (VEGF). El método induce la mitosis celular, la miocardiogénesis y la angiogénesis, arteriogénesis, vasculogénesis, linfangiogénesis en tejidos de mamíferos. El método utiliza un vector plasmídico para el transporte de la secuencia de nucleótidos codificante. La administración se realiza por vía intramiocárdica.

Figura más representativa N°

CA ARGENTINA

I.N.P.I.

SOLICITUD DE:

PATENTE DE INVENCION: X

CERTIFICADO DE MODELO DE UTILIDAD:

Fecha de Presentación

CUMBO
ULISA INGLES
5 2473
Cap. Fed.

I. Solicitante

Acta Nº:

1) Apellido y Nombre / Denominación o Razón Social: P 0101 09 2313

BIO SIDUS S.A. / FUNDACION UNIVERSITARIA Dr. RENE G. FAVALORO

MESA DE ENTRADAS MAY 15 09 I.N.P.I.

2) Documento de Identidad:

Estado Civil:

Nupcias:

Nombre del Cónyuge:

3) Caja de Jubilación o AFJP:

Nº de CUIL o CUIT: CUIT 30-59811709-4 IVA: RESP. INSCRIPTO

4) Inscripto en el Registro Industrial de la Nación (Decreto-Ley 19.971/72) Nº: No corresponde

5) Domicilio Real: Constitución 4234 -Ciudad de Buenos Aires - AR- / Solís 453 - Ciudad de Buenos Aires - AR-

Legal: Alsina 971 1° piso, of "10" – (C1088AAA) Ciudad de Buenos Aires

II. Objeto

6) Título de la Invención: METODO PARA INDUCIR LA PROLIFERACION NEOVASCULAR Y REGENERACION TISULAR

7) Carácter de la Patente:

a) Definitiva, por el término de VEINTE años

b) Adicional a la Patente Nº

8) Ley 17.011. Fecha Prioridad:

País

Nº

III. Documentación acompañada

9) Se acompaña:

a) Comprobante pago servicio requerido ☐

b) Formulario anexo en duplicado ☐

c) Carátula en duplicado ☐

Instituto Nacional de la Propiedad Industrial

PATENTE DE INVENCION

Bajo el Acta Nro. P 0101023 13

Se ha dado entrada a una solicitud de PATENTE DE INVENCION.

Buenos Aires, ... de 2001.

.....
MESA DE ENTRADAS

TRADUCCIÓN PÚBLICA.

PUBLIC TRANSLATION.

Descriptive Memory

Of the Invention Patent

On

A Method to Induce Neovascular Proliferation and Tisular Regeneration

Applied by

Bio Sidus S. A. and Fundación Universitaria Dr. René G. Favaloro.

For the term of 20 years.

There follow the logotypes corresponding to BIO SIDUS and UNIVERSIDAD FAVALORO – Buenos Aires – Argentina.

A Method to Induce Neovascular Proliferation and Tisular Regeneration

Technical description of the invention

The present invention relates to a method to induce neovascular proliferation and tisular regeneration using the vascular endothelium growth factor (VEGF). Particularly, a method for *in vivo* localized induction of neovascular proliferation and tissue regeneration in mammals through the use of VEGF.

Technical field of the invention

This invention refers to a method for stimulating revascularization and tisular regeneration.

Background of the Invention

The ischemic cardiopathy is the main cause of morbidity and mortality. The epidemiological and socioeconomical impact of the coronary heart disease is remarkable. In 1990 this pathology caused about 6.3 million deaths worldwide. *See* Murray *et al, Lancet, 349*:269-276 (1997). Developing countries are particularly affected by this disease showing a relative excess of 70% if compared to developed countries. *See* Reddy, *et al., Circulation, 97*:596:601 (1998). In Argentina, ischemic cardiopathy is the first mortality cause, with an incidence of approximately 30%, trend which tends to remain stable since 1980. For the etary group older than 65 years, this rate reaches almost 40%. *See* programa Nacional de Estadisticas de Salud (National Program of Health Statistics), Series 5, Number 38 (1994).

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APPLICATION OF-----
INVENTION PATENT: X
MODEL OF UTILITY CERTIFICATE-----
FILING DATE: -----
Proceedings No. P010102313.-----
May 15, 200-15-00-----
Reception Desk-----
I. Applicant -----
1) Name and Surname / Company's Name:-----
BIO SIDUS S.A./FUNDACIÓN UNIVERSITARIA DR. RENÉ G. FAVALORO.----
2) Identity Document:-----
Marital Status: -----
Marriage: -----
Spouse's Name: -----
3) Retirement Account or Pension Funds Management (AFJP): -----
No of CUIL (Unique Labor Identification Code) or CUIT (Unique Tax Identification Code): CUIT 30-59811709-4. ---- V.A.T: Liable for V.A.T Registration.- -----
4) Registered in the National Industrial Registry (Decree-Law 19,971/72) No.: DOES NOT APPLY- -----
5) Real Address: Constitución 4234. Buenos Aires. Argentina/Solís 453.Buenos Aires – Argentina.-----
Legal Address: Alsina 971 – First Floor, office.10 – (C1088AAA), Buenos Aires
II. PURPOSE. -----
6) Title of invention: Method to Induce Neovascular Proliferation and Tissue Regeneration.-----
7) Type of patent: -----
a) Final: for a 20-year period-----
b) Additional to Patent No.: -----
8) Act 17,011. Priority Date: -----
Country: -----
No. -----
III. Attached Documents -----

The vascular endothelium growth factor (VEGF) is a protein produced by skeletal muscle cells, smooth muscle cells, ovarian corpus luteum cells, tumor cells, fibroblasts and cardiomyocytes. Unlike other growth factors, VEGF is secreted by the cell producing it. *See* Thomas, *J. Biol. Chem.* 271:603-606 (1996); Leung, *et al., Science*, 246:1306-1309 (1989). In non-malignant tissues, VEGF human gene is expressed in four isoforms secondary to post-transcriptional alternative splicing, producing proteins with different numbers of aminoacids. To present, VEGF proteins of 121, 165, 189 and 206 aminoacids, with a molecular weight ranging from 34 to 46 kD are known. *See* Tischer, *et al., J. Biol. Chem.*, 266:11947-11954 (1991); Ferrara, *et al., J. Cell. Biochem*, 47:211-218 (1991).—VEGF specific receptors are VEGFR-1 (flt-1), VEGFR-2 (KDR/flk-1) and VEGFR-3 (flt-4). *See* Del Vries, *et al., Science*, 254:989-991 (1992): Terman, *et al., Biochem. Biophys. Res. Commun.*, 187:1579-1586 (1992); Gallant, *et al., Genomics*, 13:475-478 (1992). Due to the localization of these receptors that are apparently predominant in endothelial cells, VEGF has been described as specifically selective for these cells. VEGF bioinactivity on non-endothelial cells has been posed. *See* Jakeman, *et al., J. Clin. Invest.*, 89:244-253 (1992); Ferrara, *et al., Endocr. Rev.*, 18:4-25 (1997); Thomas, *et al., supra* (1996).——VEGF therapeutical administration represents an important challenge. VEGF can be administered as a recombinant protein (protein therapy) or by VEGF-encoding gene transfer (gene therapy). *See* Safi, *et al., J. Mol. Cell. Cardiol.*, 29:2311-2325 (1997); Simons, *et al., Circulation*, 102:E73-E86 (2000).——Protein therapy has relevant disadvantages. The extremely short mean life of the protein, conditions therapy to the administration of high or repeated doses to achieve the desired therapeutic effect. *See* Simons, *et al., supra* (2000); Takeshita, *et al., Circulation* 90:II228-234 (1994). Furthermore, intravenous administration of high doses of VEGF protein is known to produce serious refractary hypotension. *See* Henry, *et al., J. Am. Coll. Cardiol.*, 31:65A (1998); Horowitz, *et al., Arterioscl. Thromb. Vasc. Biol*, 17:2793-2800 (1997); Lopez, *et al., Am. J. Phisiol.* 273:H1317-1323 (1997). To avoid problems related to protein therapy, the use of gene therapy with genomic DNA encoding for VEGF has been described. *See* Mack, *et al., J. Thorac. Cardiovasc. Surg.* 115:168-177

synthesized for a limited period, of approximately two weeks. According to experimental studies, sustained expression during this limited period is necessary and sufficient to trigger off the angiogenic process. Based on these advantages, several research groups have studied the therapeutic effects of gene therapy using angiogenic factors in experimental models of heart and limb ischemia. These approaches have yielded positive results. *See* Magovern, *Ann. Thorac. Surg.*, *62*:425-434 (1996); Mack, *et al., supra* (1998); Tio, *et al., supra* (1999); Walder, *et al., J. Cardiovasc. Pharmacol.*, *27*:91-98 (1996); Takeshita, *et al., Lab. Invest.*, *75*:487-501 (1996); Mack, *et al., Gen. Vasc. Surg.*, 27:699-709 (1998); Tsurumi, *et al., Circulation, 94*:3281-3290 (1996).

Gene therapy has proved to achieve the desired effect without the inconveniences related to protein therapy. However, adenoviral gene therapy may induce inflammatory or systemic immune reactions, especially when administered in repeated doses. This represents a significant limitation to this kind of therapy. *See* Gilgenkrantz, *et al., Hum. Gene Ther.*, *6*:1265-1274 (1995); Dewey, *et al., Nat. Med.*, *5*:1256-1263 (1999); Werston, *et al., J. Virol.*, *72*:9491-9502 (1998); Hollon, *Nat. Med.*, 6:6 (2000), Chang, *et al., Nat. Med.*, *5*:1143-1149 (1999); Byrnes, *et al., J. Nerosci.*, *16*:3045-3055 (1996). According to recent studies, plasmid gene therapy does not have these disadvantages and can be administrated safely in repeated doses. *See* Simons, *et al., supra* (2000).

VEGF systemic administration has been associated to the potential risk of inducing undesired angiogenesis in peripheral tissues. *See* Folkman, *Nat. Med.*, *1*:27-31 (1995); Liotta, *et al., Cell, 64*:327-336 (1991); Lazarous, *et al., Circulation, 94*:1074-1082 (1996); Ferrara, *Breast Cancer Res. Treat.*, *36*:127-137 (1995); Ferrara, *Lab. Invest.*, *72*:615-618 (1995); Aiello, *et al., N. Eng. J. Med.*, *331*:1480-1485 (1994); Adams, *et al., Am. J. Ophthalmol.*, *118*:445-450 (1994); Inoue, *et al., Circulation, 98*:2108-2116 (1998); Simons, *et al., supra* (2000). Regarding site specificity and lower risk of systemic exposure, it is under discussion which kind of therapy is more efficient. These effects are probably more related to the route of administration than to the nature of the therapy. It has been suggested that local administration diminishes the risk of undesired

adaptative advantage since it is involved in the vasomotor tone regulation. Vascular smooth muscle maintains a basal vascular tone and permits self-regulation upon variations on blood flow and pressure. It has been suggested that the absence of smooth muscle layer is related to vessel collapse. *See* "Angiogenesis and Cardiovascular Disease", Ware, Ed. (Oxford University Press Inc., New York, USA., 1999), p. 258-261.

Acute myocardial infarction is consequent to coronary heart disease with the worst short and long-term prognosis. *See* Bolognese, *et al., Am. Heart J., 138*:S79-83 (1999); Mehta, *et al., Herz, 25*:47-60 (2000); Hessen, *et al., Cardiovasc. Clin., 20*:283-318 (1989); Jacoby, *et al., J. Am. Coll. Cardiol., 20*:736-744 (1992); Rosenthal, *et al., Am. Heart J., 109*:865-876 (1985). This condition results frequently in a significant loss of myocardial cells, reducing the myocyte mass. It is known in the art that cardiomyocytes of human and human-like species like pigs, preserve their ability to replicate DNA. Nevertheless they seldom multiply generating daughter cell. In most cases, these cells are not capable of progressing in the cell cycle and entering into M (mitotic) phase. *See* Kajsutra, *et al., Proc. Natl. Acad. Sci. USA, 95*:8801-8805 (1998); Pfizer *et al., Curr. Top. Pathol., 54*:125-168 (1971).

The inability of cardiomyocytes to replicate properly precludes the necessary replacement of myocardial tissue in upper animal species. Consequently, myocardial function is diminished because the infarcted area is replaced by fibrotic tissue without contractile capacity. In addition, the cardiomyocytes become hypertrophic and develop polyploid nuclei. *See* Herget, *et al., Cardiovasc. Res. 36*:45-51 (1997); "Textbook of Medical Physiology", 9th Ed., Guyton *et al.*, Eds. Mc Graw-Hill Interamericana, México, 1997), p. 276-279, 280-284.

Attempts have been made to restore myocardiocytes loss with other cells, as autologous sattelite cells and allogenic myoblasts. To present, these attemps have not been much successful. *See* Dorfman, *et. al., J. Thorac. Cardiovasc. Surg., 116*:744-751 (1998); *Murry, et. al., J. Clin. Invest., 98*: 2512-2523 (1996); *Leor, et. al., Circulation, 94: Suppl.* II-332-II-336 (1996); *Ren-Ke, et. al., Circ. Res., 78*: 283-288 (1996).

tissue loss with autologous myocardial tissue and would increase myocardial perfusion, diminishing the morbidity and mortality rates associated to left ventricular remodelling and ischemic heart disease. *See* Bolognese, *et al., supra* (1999).

Lack of mitotic capacity precludes the increase of the number of cells (hyperplasia) in front of other noxas. In these cases, the adaptive response of human and porcine myocardiocytes is the cell volume increase. Therefore, in certain pathologies (for instance hypertensive hypertrophy, dilated caediomyopathy, etc.) myocardiocytes are also markedly hypertrophic and polyploid. *See* Pfizer, *Curr. Top. Pathol., 54*:125-168 (1971); Adler, *et al., J. Mol. Cell. Cardiol., 18*:39-53 (1986). In most cases, this cell adaption is not enough. Besides, progressive myocardiac hypertrophy increases oxigen and nitrogen cell demand, thus reducing subendocardial perfusion, even in absence of coronary occlusion. Finally, the combination of these factors leads to myocardial function detriment. *See* "Textbook of Medical Physiology", 9th Ed, *supra*. P. 276-279, 307-308 An ideal method should induce complete mitosis of these hypertrophic and polyploid cells resulting in smaller and better-perfused daugther cells thus reducing the progression of cardiomyopathy towards heart failure.

Diagrams.

Fig. 1 illustrates the stress tolerance index. Pre and post-treatment mean values for Group I-T (VEGF) and Group I-P (Placebo) are compared. It is observed that Group I-T post-treatment value is higher than the same group pre-treatment value and than Group I-P pre and post-treatmnet values. Paired comparisons show: 1) absence of statistically significant differences between pre and post-treatment indexes for Group I-P and 2) presence of statistically significant differences between pre and post-treatment indexes for Group I-T. Non-paired comparisons between groups show: 1) absence of statistically significant differences between pre-treatment indexes for Group I-T and Group I-P and 2) presence of statistically significant differences between post-treatment indexes for Group I-T and Group I-P.

Fig. 2 illustrates the perfusion improvement index. Mean values for Group I-T (VEGF) and Group I-P (placebo) are compared. The value for Group I-T is

value for Group I-T (VEGF) is significantly higher than the value for Group I-P (placebo).

Fig. 5 shows the mitosis density mean value for the area under risk. The value for Group I-T (VEGF) is significantly higher than the value for Group I-P (placebo).

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Fig. 6 represents the genomic DNA transcription curve (mRNA) for Group I-T individuals estimated by RT-PCR.

Fig. 7 illustrates the metaphase of a cardiomyocyte from a Group I-T individual. The arrow points at the condensed chromosome aligned on the metaphase plate and the mitotic spindle..

Fig. 8 illustrates the telophase of a cardiomyocyte from a Group I-T individual. Sarcomeric striations are clearly visible.

Fig. 9 illustrates the mitotic process of two adjacent cardiomyocytes. The boundary between the cardiomyocytes is distinguishable. The integrity of both cardiomyocytes is clearly observed.

Figs. 10 and 11 illustrate blood vessels with smooth muscle layer proliferation (angiogenesis) in myocardial tissue. Vascular smooth muscle was identified with alpha-actin immunohistochemical stain.

Deposit.

Plasmid pUVEK15 was deposited on November 13, 2000, under access number DSM 13833 at the DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen, Mascheroder Weg 1B, D-38124 Braunschweig, Federal Republic of Germany.

Description of the Invention

One advantage of the present invention is the secure and efficient induction of neovascular proliferation in hypoperfused and normoperfused tissues. By utilizing the claimed method, it is possible to stimulate the neoformation and growth of vessels and smooth and striated muscular cells. The method is particularly useful for inducing revascularization in patients with ischemic heart disease. The claimed method is characterized for the absence of adverse side effects related to the systemic exposure to angiogenic factors in high doses.

Another advantage of the present invention is the regeneration of myocardial

Another advantage of this invention is its potential use to effectively and efficiently revascularizate the myocardyum in transplanted patients with chronic graft rejection and diffuse coronary disease. The claimed method would restore the impaired perfusion in these patients who are frequently not eligible for conventional revascularization methods.

An additional potential advantage of the present invention is its use for increasing perfusion in ischemic tissues of patients with diabetes-related micro and macroangiopathy. The claimed method may reduce chronic complications associated to diabetes such as diabetic neuropathy, ischemic heart disease, peripheral artery disease and severe limb ischemia, among others. *See Schratzberger, et al., J. Clin. Invest., 107*:1083-1092 (2001); Rivard, *et al., Circulation 96 Suppl I:* 175 (1997); Rivard, *et al., Am. J. Pathol., 154*: 355-363 (1999).

One of the advantages of the claimed method is its higher safety when used along with minimally invasive procedures of percutaneous intramyocardial-transendocardial administration. This administration is achieved by accessing to the left ventricular chamber through a catheter mediated endovascular approach. This type of administration may be assisted by an electromechanical mapping of the left ventricle. In this way the morbility and mortality associated to open-chest surgery is significantly diminished.

The present invention refers to a method for inducing neovascular proliferation and tissue regeneration characterized for the administration of a nucleotide sequence encoding for the active site of a polypeptide that includes the amino acid sequence SEQ. ID. No. 1. In an embodiment of the present invention, a nucleotide sequence encoding for a polypeptide whose amino acid sequence is SEQ ID No. 1 is administered. In another embodiment of the present invention, the active site of a polypeptide including the amino acid sequence SEQ ID No. 1 is administered. In another embodiment of the present invention, a polypeptide including the amino acid sequence SEQ ID No.. 1 is administered. The nucleotide sequence utilized according to the present invention may be genomic DNA, cDNA and messenger RNA. Preferably, the nucleotide sequence is genomic DNA.

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skeletal striated muscle cells type I and type II, vascular smooth muscle cells and non-vascular smooth muscle cells and myoepithelial cells. More preferably, the muscle cells utilized are cardiomyocytes.

The claimed method is characterized for inducing vascular proliferation. Preferably, the induced vascular proliferation is localized in the site of administration of the inducing agent. More preferably, the site of administration is the myocardium.

The claimed method is characterized for inducing localized angiogenesis both *in vivo* and *ex vivo*. Preferably, angiogenesis is localized at the administration site of the inducing agent. More preferably, the site of administration is the myocardium. In an embodiment of the present invention, angiogenesis is induced in normoperfused tissue, either *in vivo*, *in vitro* or *ex vivo*. In another embodiment of the present invention, angiogenesis is induced in ischemic tissue, either *in vivo*, *in vitro* or *ex vivo*. Preferably, the claimed method induces angiogenesis in hypoperfused myocardial tissue, either *in vivo*, *in vitro* and *ex vivo*. Hypoperfused myocardial tissue may be ischemic, viable, hibernated, stunned, preconditioned, injured, infarcted, non-viable, fibrosed and necrosed. More preferably, the claimed method induces angiogenesis *in vivo* in hypoperfused myocardial tissue.

The claimed method is also characterized for inducing arteriogenesis *in vivo*, *in vitro* and *ex vivo*. Preferably, arteriogenesis is localized at the site of administration. More preferably, the site of administration is the myocardium. In an embodiment of the present invention, arteriogenesis is induced in normoperfused tissue *in vivo*, *in vitro* and *ex vivo*. In another embodiment of the present invention, arteriogenesis is induced in ischemic tissue, *in vivo*, *in vitro* and *ex vivo*. Preferably, the claimed method induces arteriogenesis in hypoperfused myocardial tissue *in vivo*, *in vitro* and *ex vivo*. Hypoperfused myocardial tissue may be ischemic, viable, hibernated, stunned, preconditioned, injured, infarcted, non-viable, fibrosed and necrosed. More preferably, the claimed method induces arteriogenesis in hypoperfused myocardial tissue *in vivo*.

The claimed method is also characterized for inducing vasculogenesis *in vivo*, *in*

myocardial tissue may be ischemic, viable, hibernated, stunned, preconditioned, injured, non-viable, infarcted, necrosed and fibrosed. More preferably, the claimed method induces vasculogenesis in hypoperfused myocardial tissue *in vivo*.

The claimed method is also characterized for inducing lymphangiogenesis *in vivo*, *in vitro* and *ex vivo*. Preferably, lymphangiogenesis is localized in the site of administration. More preferably, the site of administration is the myocardium. In an embodiment of the present invention, lymphangiogenesis is induced in normoperfused tissue, *in vivo*, *in vitro* and *ex vivo*. In another embodiment of the present invention, lymphangiogenesis is induced in ischemic tissue, *in vivo*, *in vitro* and *ex vivo*. Preferably, the claimed method induces lymphangiogenesis in hypoperfused myocardial tissue, *in vivo*, *in vitro* and *ex vivo*. Hypoperfused myocardial tissue may be ischemic, viable, hibernated, stunned, preconditioned, injured, non-viable, infarcted, necrosed and fibrosed. More preferably, the claimed method induces lymphangiogenesis in hypoperfused myocardial tissue *in vivo*.

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The claimed method is also characterized for inducing mitosis *in vivo*, *in vitro* and *ex vivo*. Preferably, mitosis is induced locally at the site of administration. More preferably, the site of administration is the myocardium. In an embodiment of the present invention, mitosis is induced in normoperfused tissue, *in vivo*, *in vitro* and *ex vivo*. In another embodiment of the present invention, mitosis is induced in ischemic tissue *in vivo*, *in vitro* and *ex vivo*. Preferably, the claimed method induces mitosis in hypoperfused myocardial tissue, *in vivo*, *in vitro* and *ex vivo*. Hypoperfused myocardial tissue may be ischemic, viable, hibernated, stunned, preconditioned, injured, non-viable, infarcted, necrosed and fibrosed. More preferably, the claimed method induces mitosis in hypoperfused myocardial tissue *in vivo*.

The claimed method is also characterized for inducing tissue regeneration *in vivo*, *in vitro and ex vivo*. Preferably, tissue regeneration is induced locally in the site of administration. More preferably, the site of administration is the myocardium. In an embodiment of the present invention, tissue regeneration is induced in normoperfused territory, *in vivo*, *in vitro* and *ex vivo*. In another

In an embodiment of the present invention, the codifying nucleotide sequence is operably linked to a vector. In an embodiment of the claimed method, the vector is a viral vector such as adenovirus, adeno-associated virus, retrovirus and lentivirus. In another embodiment of the present method, the vector is a plasmid vector. More preferably, the plasmid vector is pUVEK15. In another embodiment of the present invention, the nucleotide sequence is transported by a liposome. In an embodiment of the present invention, the inducing agent is contained in a proper pharmaceutical compound. The pharmaceutical compound containing the inducing agent is administered to the receptor in sufficient doses.----------

The pharmaceutical compound used according to the present invention may be administered by intravenous, intracoronary, intra-aortic, intrafemoral, intrapopliteal, intrapedialis, intra-posterior tibialis, intracarotideal and intraradialis routes. The pharmaceutical compound may be also administered by intrapericardial, intraperitoneal, intra-amniotic, intrapleural, intramyocardial-transepicardial, intramyocardial-transendocardial, intra-peripheral muscle, subcutaneous, intraspinal, and intracardiac (intra-atrial and intraventricular) routes. In addition, the inducing agent may be administered by sublingual, inhalatory, oral, rectal, periadventitial, perivascular, topical epicardial, topical epidermal, transdermal, ophthalmic routes or through the conjunctival, nasopharyngeal, bucopharyngeal, laryngopharyngeal, vaginal, colonic, urethral and vesical mucoses. Preferably, the inducing agent is administered by intramyocardial-transepicardial and intramyocardial-transendocardial injections. More preferably, the inducing agent is administered by intramyocardial-transepicardial injection.----------

In an embodiment of the present invention, the inducing agent is preferably injected perpendicular to the plane of injection area. In another embodiment of the present invention, the inducing agent is injected in parallel to the plane of the area of injection. In another embodiment of the present invention, the inducing agent is injected in an oblique angle in relation to the plane of the injection area. Preferably, injections are homogeneously distributed in the area of injection.----

As used herein, "inducing agent" is defined as genomic DNA, cDNA or messenger RNA encoding for the VEGF active site. "Inducing agent" also

As used herein, "area under risk" means the myocardial area irrigated by the circumflex coronary artery.

As used herein, "arteriogenesis" is defined as the development of blood vessels with smooth muscle media layer.

As used herein, the term "underdifferentiated cells" is defined as any pluripotent cell with the capacity of originating cells with a different phenotypic profile, but at the same time they may originate other pluripotent cell. These cells show characteristics that are different from all known cell profiles constituing adult tissues. "Underdifferentiated cells" include, but are not limited to, stem cells, mesenchymatous cells, hemangioblasts, angioblasts, and hematopoietic precursor cells.

As used herein, the term "underdifferentiated cells" is defined as any cell having characteristics which are inherent to its phenotypic profile but which at the same time may originate cells having a different phenotypic profile. "Underdifferentiated cells" includes, but are not limited to, fibroblasts, myoblasts, osteoblasts, precursor endothelial cells, skeletal muscle satellite cells, and neural tissue glyal cells.

As used herein, "paired comparison" refers to the statistical comparison of the same group of individuals at different evolutive times.

As used herein, "non-paired comparison" refers to the statistical comparison between two different groups of individuals at the same time.

As used herein, "pharmaceutical compound" refers to a solvent, adjuvant or excipient used to administrate an inducing agent. "Pharmaceutical compound" includes any solvent, dispersion media, aqueous, gaseous solutions, antibacterial and antifungal agents, isotonic agents, either absorption delayer or accelerator agents, or similar substances. The use of said substances in the administration of pharmaceutical active compounds is known in the art. Except when a conventional substance or agent is not compatible with the inducing agent, its use in the pharmaceutical compounds is contemplated. Supplementary active ingredients may also be incorporated to the pharmaceutical compound utilized in the present invention. "Pharmaceutical compounds" include, but are not limited to, inert solid fillings or solvents, sterile aqueous solutions and several

cases, the formulation should be sterile. The formulation may be fluid to facilitate syringe dispensation. The formulation should also be stable under manufacturing and storage conditions and should be preserved against the contaminant action of microorganisms such as bacteria, viruses and fungi.

As used herein, "length density index" refers to a vascularization parameter for a hystopathologically studied tissue, according to a methodology already described. This parameter was designed to quantify vessels arranged in any variety of orientation. The method for calculating this index is known in the art. *See* Anversa *et al., Am. J. Physiol., 260:* H1552-H1560 (1991); Anversa *et al., Am. J. Physiol., 267:* H1062-H1073 (1994).

As used herein, "mitotic density" refers to the quotient of the number of mitosis per 10^6 cardiomyocyte nuclei.

As used herein, "sufficient dose" is defined as a quantity of the inducing agent, or of the pharmaceutical compound including the inducing agent, adequate to attain the specified function. In the context of the present invention, "sufficient dose" refers to a quantity of the inducing agent, or of the pharmaceutical compound including the inducing agent, adequate to produce one or more of the following results: the induction of angiogenesis, arteriogenesis, vasculogenesis, lymphangiogenesis or mitosis in eukaryotic cells.

As used herein, "stress tolerance index" is defined as the arithmetical difference between the percentual perfusion value (stress) and the percentual perfusion value at rest. This index is calculated in post-treatment and pre-treatment situations.

As used herein, "perfusion improvement index" refers to the arithmetical difference between the post-treatment stress tolerance index and the pre-treatment stress tolerance index.

As used herein, "post-treatment stress tolerance index" is defined as the arithmetical difference between the post-treatment perfusion value during pharmacological challenge (stress) and the post-treatment percentual perfusion value at rest.

As used herein, "pre-treatment stress tolerance index" is defined as the arithmetical difference between the pre-treatment perfusion value during

VEGF as a vascular proliferation stimulator.

As used herein, "lymphangiogenesis" is defined as the development or proliferation of lymphatic vessels.

As used herein, "localized" is defined as the restriction of the response of an inducing agent to the area of interest.

As used herein, "mammal" is defined as a hot blooded vertebrate animal whose progeny is fed with milk secreted by its mammal glands. The term "mammal" includes, but is not limited to, rats, mice, rabbits, dogs, cats, goats, sheep, cows, pigs, primates and humans.

As used herein, "mitosis" refers to the cell division process.

As used herein, "neovascular proliferation" is defined as an increase of a tissue vascularization due to the expansion of the existing vascular bed or to the formation of new vascular beds. Neovascular proliferation includes angiogenesis, arteriogenesis, vasculogenesis and lymphangiogenesis.

As used herein, "vasculogenesis" is defined as the vascular development of blood vessels derived from undifferentiated or underdifferentiated cells.

As used herein, "VEGF" is defined as any vascular endothelial growth factor. "VEGF" includes, but is not limited to, the VEGF variants A, B, C, D, E and F. *See* Hamawy, *et al., Curr. Opin. Cardiol., 14*:515-522 (1999); Neufeld, *et al., Prog. Growth Factor Res., 5*:89-97 (1994); Olofsson, *et al., Proc. Natl. Acad. Sci. USA, 93*:2576-2581 (1996); Chilov, *et al., J. Biol. Chem., 272*:25176-25183 (1997); Olofsson, *et al., Curr. Opin. Biotechnol., 10*:528-535 (1999). The VEGF A variant includes, but is not limited to, isoforms $VEGF_{1-121}$, $VEGF_{1-145}$, $VEGF_{1-189}$ and $VEGF_{1-206}$. The SEQ ID No. 1 illustrates an example of isoform $VEGF_{1-165}$. *See* Tischer, *et al., J. Biol. Chem., 266*:11947-11954 (1991); Poltorak, *et al., J. Biol. Chem., 272*:7151-7158 (1997). The term "VEGF" also includes the vascular permeability factor or vasculotropin (VPF). *See* Keck, *et al., Science 246*:1309-1312 (1989); Senger, *et al., Science*, 219:983-985 (1983). VPF is currently known in the art as VEGF A.

The present invention uses a plasmid called pUVEK15 of approximately 3068 base pairs (bp). The pUVEK15 plasmid is characterized for including a citomegalovirus (CMV) promoter, a chimeric intron, a DNA fragment containing

Having described the invention in general terms, it will be more easily understood by reference to the following examples which are presented as an illustration and are not intended to limit the present invention, save when specifically indicated.–

Example 1

Induction of Ischemia

Eighty Landrace pigs weighing approximately 25 kg (aaround 3 months of age) were submitted to the following protocol: 1) each individual underwent clinical and laboratory assessment of good health; 2) a sterile thoracotomy was performed at the 4th left intercostal space under general anesthesia (induction: thiopental sodium 20 mg/kg; maintenance: 2% enflurane) and the circumflex coronary artery was dissected free from surrounding tissue at its proximal portion; 3) an Ameroid constrictor was placed embracing the origin of the circumflex coronary artery; and 4) the thoracotomy was repaired.

Example 2

Basal Pre-treatment Studies

Three weeks after the first surgery indicated in the previous example, basal (pre-treatment) studies were performed on the individuals. The studies were conducted under sedation with sufficient doses of intravenous sodium thiopental and under electrocardiographic control. Basal myocardial perfusion studies were performed on each individual. The left ventricular perfusion was quantified by single photon emission computed tomography (SPECT) utilizing an ADAC Vertex Dual Detector Camera System (ADAC Healthcare Information Systems Inc., USA). Sestamibi marked with Tc^{99}.

The studies were performed at rest and under pharmacological challenge with progressive doses of intravenous dobutamine. The dobutamine infusion was interrupted when heart rate was at least a 50% above the basal (rest) values.

Individuals fulfilling the inclusion criterium (hipoperfusion in a territory consistent with the circumflex coronary artery bed) were selected. From the subjects considered, only twenty six individuals developed chronic myocardial ischemia and were selected as satisfying the inclusion criterium.

Example 3.

Administration of VEGF Plasmid and Placebo Plasmid.

The treated group was designated Group I-T. The placebo group was designated Group I-P.

Group II individuals were randomized into two subgroups (Group II-T and Group II-P). Eight individuals were allocated to Group II-T. Two individuals were allocated to Group II-P. The treated group was designated Group II-T. The placebo group was designated Group II-P.

A sterile reopening of the previous thoracotomy was performed on each individual from both Group I and Group II under general anesthesia (induction: sodium thiopental 20 mg/kg, maintenance: 2% enflurane).

Each individual from Groups I-T and II-T received 10 injections and 3 aliquots respectively of a solution contalning pUVEK15 plasmid encoding for vascular endothelial growth factor (1.9 mg of pUVEK15 in 1 mL of saline). Each injection contained 200 µl of the plasmid solution.

Each individual from Groups I-P and II-P received 10 injections and 3 aliquots respectively of a solution containing pUVEK15^{-VEGF} plasmid without the encoding region for the vascular endothelial growth factor (1.9 mg of pUVEK15^{-VEGF} in 1 mL of saline solution). Each injection contained 200 µl of the plasmid solution.

Each aliquot was injected intramyocardically. The area of injection included the hypoperfused zone, the transition zone and the normoperfused tissue immediately surrounding the transition zone. The injections were administered at a 45 degree angle in relation to the plane of the myocardium area, avoiding intraventricular administration of the inducing agent The injections were homogeneously distributed in the area of injection. The thoracotomy was repaired in each individual after administration.

Example 4.

Post-treatment Studies

1. Histopathological and physiological studies.

Five weeks after the second surgery (reoperation), post-treatment studies were performed on Group I individuals. The individual were sedated with sufficient doses of intravenous sodium thiopental. The left ventricular perfusion was assessed for each individual following the protocol described in example 2..

Afterwards, the individuals were euthanized. The kidneys, liver, lungs, skeletal

were mounted on slides previously wetted in a 0.01% polylysine aqueous solution (Sigma Chemical Co., U.S.A.) and dried at 37° C. The sections were stained with hematoxylin-eosin and Gomori trichromic solution.

Identification of smootht layer muscle intramyocardial vessels (arteriogenesis) was performed under immunohistochemistry employing monoclonal antibodies against alpha-actin (Biogenex Labs. Inc., U.S.A.) Regarding intramyocardyal collateral vessels, only those ranging from 8 to 50 µm of maximum diameter were considered for both subgroups of individuals. For quantification of the length density of relevant intramyocardyal vessels, a digital analysis system was employed (Vidas Kontron, Germany). In addition, the length density for intramyocardyal vessels ranging from 8 to 30 µm was analyzed. Peripheral tissues were submited to a general hystopathologic analysis to determine angiogenesis and other adverse effects.

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To evidence mitosis in the myocardyal tissue, immunohistochemistry with monoclonal antibodyies against the Ki67 antigen (Novocastra Labs., U.K.).was performed in Group I. The Ki67 is a protein expressed during the whole cell cycle except for G0, and G1 early phases. The Ki67 expression pattern is not affected by DNA damage or by apoptosis induction. *See* Brown *et al., Histopathology, 17*:489-503 (1990); Gerdes *et al., J. Immunol., 133*:1710-1715 (1984); Ross *et al., J. Clin. Pathol., 48*:M113-117 (1995). Striated cells whose chromosomes presented the Ki67 antigen markedly stained, were identified as cardiomyocytes undergoing mitotic process. For the determination of the proportion of cardiomyocytes undergoing mitosis process, the number of cardiomyocytes nuclei and mitotic events in the myocardiac tissue were quantified and mitosis density for each individaul was determined.

2. Presence and Expresion of VEGF plasmid.

After the second surgery (reoperation) the Group II individuals were euthanized, according to the following chronogram: 2 individuals from Group II-T after 3 days of reoperation, 2 individuals from Group II-T and 2 individuals from Group II-P after 10 days of reoperation, 2 individuals of Group II-T after 16 days of reoperation and 2 individuals from Group II-T after 35 days of reoperation. Necropsies were performed in each euthanized individual. Myocardial tissue of

al., Nucleic. Acids Res., 17:2919 (1989). Presence of the protein (human VEGF) was evidenced by immunochemistry employing monoclonal antibodies against human VEGF (Biogenex Labs, Inc., USA) ———————————

Results.———————————

Histopathological and physiological analysis.———————————

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The perfusion and histopathological studies showed vascular proliferation in the myocardial tissue of treated individuals. The histopathological study also revealed the induction of mitosis in cardiomyocytes, endothelial cells and smooth muscle cells of Group I-T individuals.———————————

The stress tolerance index and perfusion improvement index were determined for each myocardial segment of all Group I individuals. Segments of the area under risk were studied. Then the mean values of these myocardyal tissues were calculated, in such a way that a sole value of the area under risk for each indexx in each individual was obtained Finally, the indexes obtained in each individual were averaged to obtain the values corresponding to Groups I-T and I-P.———

In this way, the analysis of the perfusion study revealed that:———————

a) Group I-P: absence of statistically significant differences between the pre-treatment and post-treatment stress tolerance indexes (paired comparison). This result indicates that there were no significant changes regarding perfusion in the placebo group, after treatment.———————————

b) Group I-T: post-treatment stress tolerance index significantly higher than pre-treatment stress tolerance index (paired comparison). This result indicates that perfusion improved significantly in the Group I-T individuals after treatment.———

c) Pre-treatment stress tolerance indexes: absence of statistically significant differences between Group I-T individuals and Group I-P individuals (non-paired comparison). This result demonstrates that perfusion was homogenous for both subgroups before treatment.———————————

d) Post-treatment stress tolerance indexes: Group I-T index was significantly higher than Group I-P value (non-paired comparison). This result indicates that after treatment, Group I-T individuals showed a better stress tolerance than Group I-P individuals .———————————

e) Perfusion improvement indexes: The mean value for Group I-T individuals

the perfusion and stress tolerance of Group I-T individuals. *See* Tables 1 and 2; Figs. 1 and 2.

The histopathological study showed statistically significant differences in length density and mitotic density indexes between both subgroups. The Group I-T individuals presented higher mean values for these indexes when compared to Group I-P individuals. *See* Tables 3, 4 and 5; Figs. 3, 4, 5, 7, 8, 9, 10 and 11. These results confirmed neovascular proliferation in the tissue of the group of individuals treated *in vivo*. Vascular proliferation evidently implies an increase in the number of cells forming part of these neovessels (endothelial and vascular smooth muscle cells). *See* Figs. 10 and 11. Therefore, the administration of the inducing agent enhanced mitosis of vascular cells in the individuals treated. Additionally, the group of individuals treated with the inducing agent showed a proportion of cardiomyocytes in mitotic process more than 5 times higher than the control group. *See* Figs. 5, 7 8 and 9; Table 5.

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Angiogenesis or other adverse side effects were not detected in the peripheral tissues of the treated individuals (Group I-T).

2. Presence and Expresion of the VEGF Plasmid

Molecular studies showed presence of plasmid DNA in injected myocardial tissue of all individuals. Plasmid DNA encoding for VEGF was found in the studied tissue of the Group II-T individuals. Placebo plasmid DNA was found in the studied tissue of the Group II-P individuals.

A transcription curve (presence of mRNA) showing a peak by day 10 post-reoperation was obtained in the Group II-T individuals. *See* Fig. 6; Table 6. Presence of mRNA in group II-P was negative.

Immunohistochemistry revealed presence of protein in cardiomyocytes and in myocardyal insterstitial cells in Group I-T individuals (day 35). In Group I-P individuals, immunohitochemistry was completely negative.

Table 1.

	Stress Tolerance Index				
	Pre-treatment (1)		Post-treatment (2)		
	Mean	σ	Mean	σ	*P* value (1) vs (2)

Table 2

	Perfusion Improvement Index	
	Mean	σ
Group I-P	-0.6	2.6
Group I-T	6.9	2.6
P value I-T vs I-P	0.058	

Table 3

	Length Density Index (8-50 µm)	
	Mean	σ
Group I-T	2.45	0.40
Group I-P	1.35	0.26
P Value I-T vs I-P	<0.04	

Table 4

	Length Density Index (8-30 µm)	
	Mean	σ
Group I-T	1.01	0.13
Group I-P	0.58	0.08
P Value I-T vs I-P	<0.02	

Table 5

	Mitotic Index	
	Mean	σ
Group I-T	113.94	24.94
Group I-P	22.49	10.58

scope of the claims of the present invention. All the publications herein quoted are included herein as references to the invention's description.

Claims

What is claimed is:

1. A method to induce neovascular proliferation and tissue regeneration characterized for the administration to a tissue of a nucleotide sequence encoding for the active site of a polypeptide characterized for comprising the amino acid sequence (SEQ ID No. 1):

Ala	Pro		Met	Ala	Glu	Gly	Gly	Gly	Gln	Asn
His	His		Glu	Val	Val	Lys	Phe	Met	Asp	Val
Tyr	Gln		Arg	Ser	Tyr	Cys	His	Pro	Ile	Glu
Thr	Leu	Val	Asp	Ile	Phe	Gln	Glu	Tyr	Pro	
Asp	Glu	Ile	Glu	Tyr	Ile	Phe	Lys	Pro	Ser	
Cys	Val	Pro	Leu	Met	Arg	Cys	Gly	Gly	Cys	
Cys	Asn	Asp	Glu	Gly	Leu	Glu	Cys	Val	Pro	
Thr	Glu	Glu	Ser	Asn	Ile	Thr	Met	Gln	Ile	
Met	Arg	Ile	Lys	Pro	His	Gln	Gly	Gln	His	
Ile	Gly	Glu	Met	Ser	Phe	Leu	Gln	His	Asn	
Lys	Cys	Glu	Cys	Arg	Pro	Lys	Lys	Asp	Arg	
Ala	Arg	Gln	Glu	Asn	Pro	Cys	Gly	Pro	Cys	
Ser	Glu	Arg	Arg	Lys	His	Leu	Phe	Val	Gln	
Asp	Pro	Gln	Thr	Cys	Lys	Cys	Ser	Cys	Lys	
Asn	Thr	Asp	Ser	Arg	Cys	Lys	Ala	Arg	Gln	
Leu	Glu	Leu	Asn	Glu	Arg	Thr	Cys	Arg	Cys	
Asp	Lys	Pro	Arg	Arg						

2. A method, according to claim 1, characterized because the tissue comprises tissue formed by eukaryotic cells.

3. A method, according to claim 1, characterized because the eukariotic cells comprise muscular cells.

4. A method, according to claim 3, characterized because the muscular cells comprise striated, smooth and myoepithelial cells.

8. A method, according to claim 1, characterized because the eukaryotic cells comprise mammalian cells.

9. A method, according to claim 8, characterized because the mammalian cells comprise porcine and human cells.

10. A method, according to claim 9, characterized because the mammalian cells are human cells.

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11. A method, according to claim 1, characterized for comprising the localized induction of vascular proliferation.

12. A method, according to claim 1, characterized for comprising the induction of localized angiogenesis *in vivo, in vitro* and *ex vivo*.

13. A method, according to claim 12, characterized for comprising the induction of angiogenesis in normoperfused tissue *in vivo, in vitro* and *ex vivo*.

14. A method, according to claim 12, characterized for comprising the induction of angiogenesis in ischemic tissue *in vivo, in vitro* and *ex vivo*.

15. A method, according to claim 12, characterized for comprising the induction of angiogenesis in myocardial tissue *in vivo, in vitro* and *ex vivo*.

16. A method, according to claim 1, characterized for comprising the induction of arteriogenesis *in vivo, in vitro* and *ex vivo*.

17. A method, according to claim 1, characterized for comprising the induction of localized arteriogenesis.

18. A method, according to claim 17, characterized for comprising the induction of arteriogenesis in normoperfused tissue *in vivo* and *ex vivo*.

19. A method, according to claim 17, characterized for comprising the induction of arteriogenesis in ischemic tissue *in vivo* and *ex vivo*.

20. A method, according to claim 17, characterized for comprising the induction of arteriogenesis in myocardial tissue *in vivo* and *ex vivo*.

21. A method, according to claim 1, characterized for comprising the induction of localized vasculogenesis *in vivo, in vitro* and *ex vivo*.

22. A method, according to claim 1, characterized for comprising the induction of localized vasculogenesis.

23. A method, according to claim 21, characterized for comprising the induction of vasculogenesis in normoperfused tissue *in vivo* and *ex vivo*.

27. A method, according to claim 1, characterized for comprising the induction of localized lymphangiogenesis.

28. A method, according to claim 26, characterized for comprising the induction of lymphangiogenesis in normoperfused tissue *in vivo* and *ex vivo*.

29. A method, according to claim 26, characterized for comprising the induction of lymphangiogenesis in ischemic tissue *in vivo* and *ex vivo*.

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30 A method, according to claim 26, characterized for comprising the induction of lymphangiogenesis in myocardial tissue *in vivo* and *ex vivo*.

31. A method, according to claim 1, characterized for comprising the induction of mitosis *in vivo, in vitro* and *ex vivo*.

32. A method, according to claim 1, characterized for comprising the induction of localized mitosis in tissue formed by eukaryotic cells.

33. A method, according to claim 31, characterized for comprising the induction of mitosis in eukaryotic cells of normoperfused tissue *in vivo, in vitro* and *ex vivo*.

34. A method, according to claim 31, characterized for comprising the induction of mitosis in eukaryotic cells of ischemic tissue *in vivo, in vitro* and *ex vivo*.

35. A method, according to claim 31, characterized by comprising the mitosis in eukaryotic cells of myocardial tissue *in vivo, in vitro* and *ex vivo*.

36. A method, according to claim 1, characterized for comprising the induction of tissue regeneration.

37. A method, according to claim 31, characterized for comprising the induction of tissue regeneration in normoperfused territories *in vivo, in vitro* and *ex vivo*.

38. A method, according to claim 31, characterized for comprising the induction of tissue regeneration in ischemic territories *in vivo, in vitro* and *ex vivo*.

39. A method, according to claim 31, characterized by comprising the induction of myocardial tissue regeneration, *in vivo, in vitro* and *ex vivo*.

40. A method, according to claim 1, characterized because the nucleotide sequence comprises genomic DNA, copy DNA and messenger RNA encoding for the active site of the polypeptide having the sequence SEQ ID No. 1.

41. A method, according to claim 1, characterized because the nucleotide sequence comprises genomic DNA encoding for the active site of the

44. A method, according to claim 43, characterized because the vector comprises a viral vector.

45. A method, according to claim 44, characterized because the viral vector comprises an adenovirus, adeno-associated viruses, retrovirus and lentivirus.

46. A method, according to claim 43, characterized because the vector comprises a plasmid vector.

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47. A method, according to claim 46, characterized because the plasmid vector is pUVEK15.

48. A method, according to claim 1, characterized because the nucleotide sequence is transported by a lyposome.

49. A method, according to claim 46, characterized because the plasmid vector is transported by a lyposome.

50. A method, according to claim 1, characterized because the nucleotide sequence is comprised in a suitable pharmaceutical compound.

51. A method, according to claim 1, characterized for comprising the administration of the pharmaceutical compound by parenteral, sublingual, inhalatory, oral and rectal routes.

52. A method, according to claim 51, characterized because the administration of the pharmaceutical compound by parenteral route comprises the intravascular, intracelomic, intramuscular, subcutaneous, intraspinal, topical and intracardiac administration.

53. A method, according to claim 52, characterized because the intravascular administration comprises the intravenous and intra-arterial administration.

54. A method, according to claim 53, characterized because the intra-arterial administration comprises the intracoronary, intra-aortic, intrafemoral, intrapopliteal, intrapedialis, intra-posterior tibialis, intracarotideal and intraradialis administration.

55. A method, according to claim 51, characterized because the intracelomic administration comprises the intrapericardial, intraperitoneal, intra-amniotic sac and intrapleural administration.

56. A method, according to claim 52, characterized because the intramuscular administration comprises the intramyocardial and intra-peripheral muscle

59. A method, according to claim 58, characterized because the administration by mucous absorption comprises the administration through the conjunctival, nasopharyngeal, bucopharyngeal, laryngopharyngeal, vaginal, colonic, urethral and vesical mucosas.

60. A method, according to claim 59, characterized because the administration by absorption through the bucopharyngeal mucosa comprises administration through yugalis, gingovoyugalis and gingivolabialis mucosas.

61. A method, according to claim 52, characterized because the intracardiac administration comprises the intra-atrial and intraventricular administration.

62. A method, according to claim 61, characterized because the intra-atrial administration comprises the intra-left atria administration and intra-right atria administration.

63. A method, according to claim 61, characterized because the intraventricular administration comprises intra-left ventricle administration and intra-right ventricle administration.

64. A method, according to claim 1, characterized for comprising the administration of the pharmaceutical compound in sufficient doses.

65. A method, according to claim 1, characterized for comprising the administration of the nucleotide sequence by intramyocardial-transepicardial injection under direct visualization.

66. A method, according to claim 1, characterized for comprising the injection of the nucleotide sequence perpendicular to the plane of the area of injection.

67. A method, according to claim 1, characterized for comprising the homogeneous injection of the nucleotide sequence in the area of injection.

68. A method, according to claim 1, characterized for comprising the administration of the active site of the polypeptide encoded by the sequence SEQ ID No. 1.

69. A method, according to claim 1, characterized for comprising the administration of the polypeptide encoded by the sequence SEQ ID No. 1.

70. The pharmaceutical compounds according to claim 50.

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lymphangiogenesis in mammalian tissues. The method employs a plasmid vector encoding the nucleotide sequence. The administration is performed by intramyocardial route.

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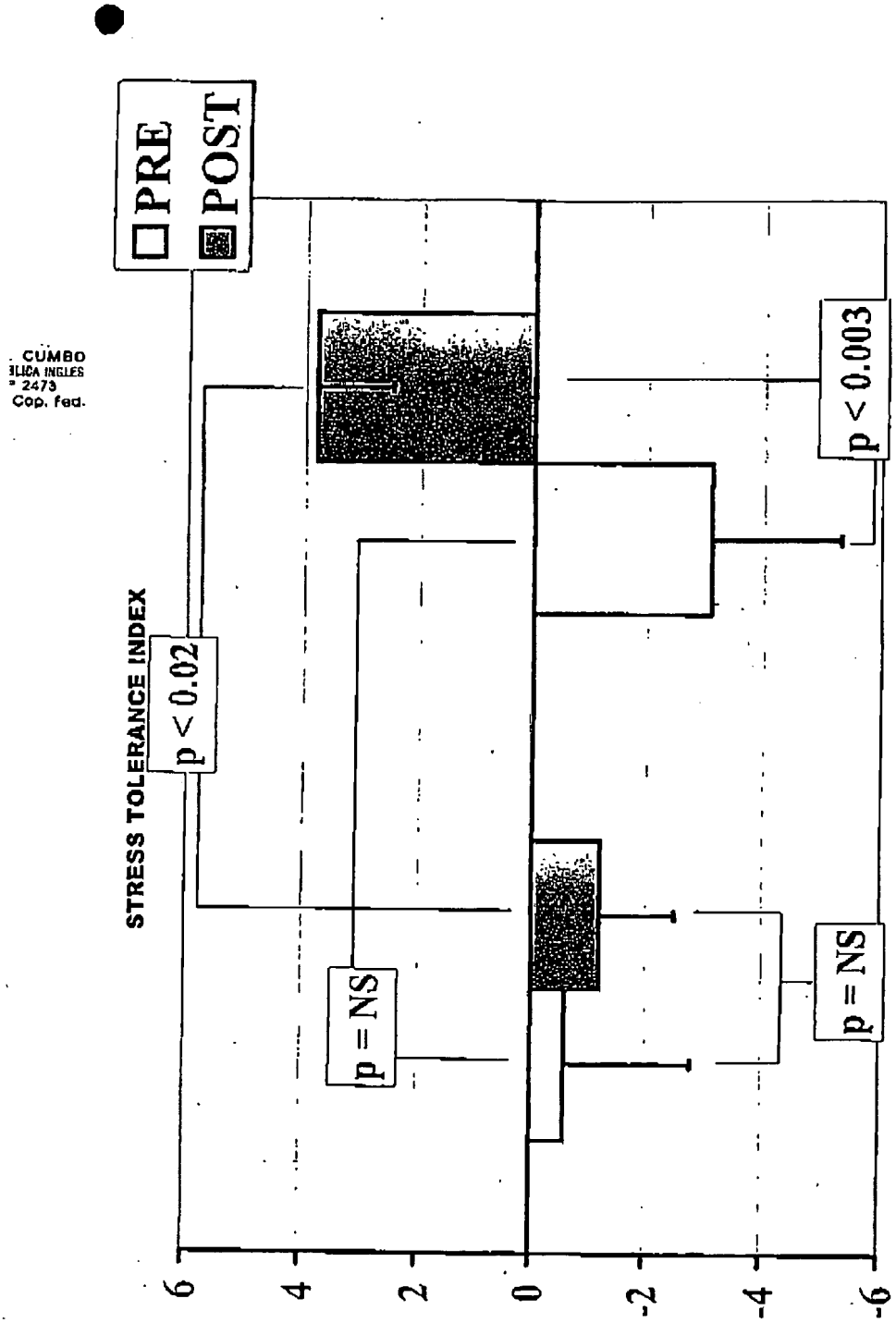
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POST
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p < 0.003
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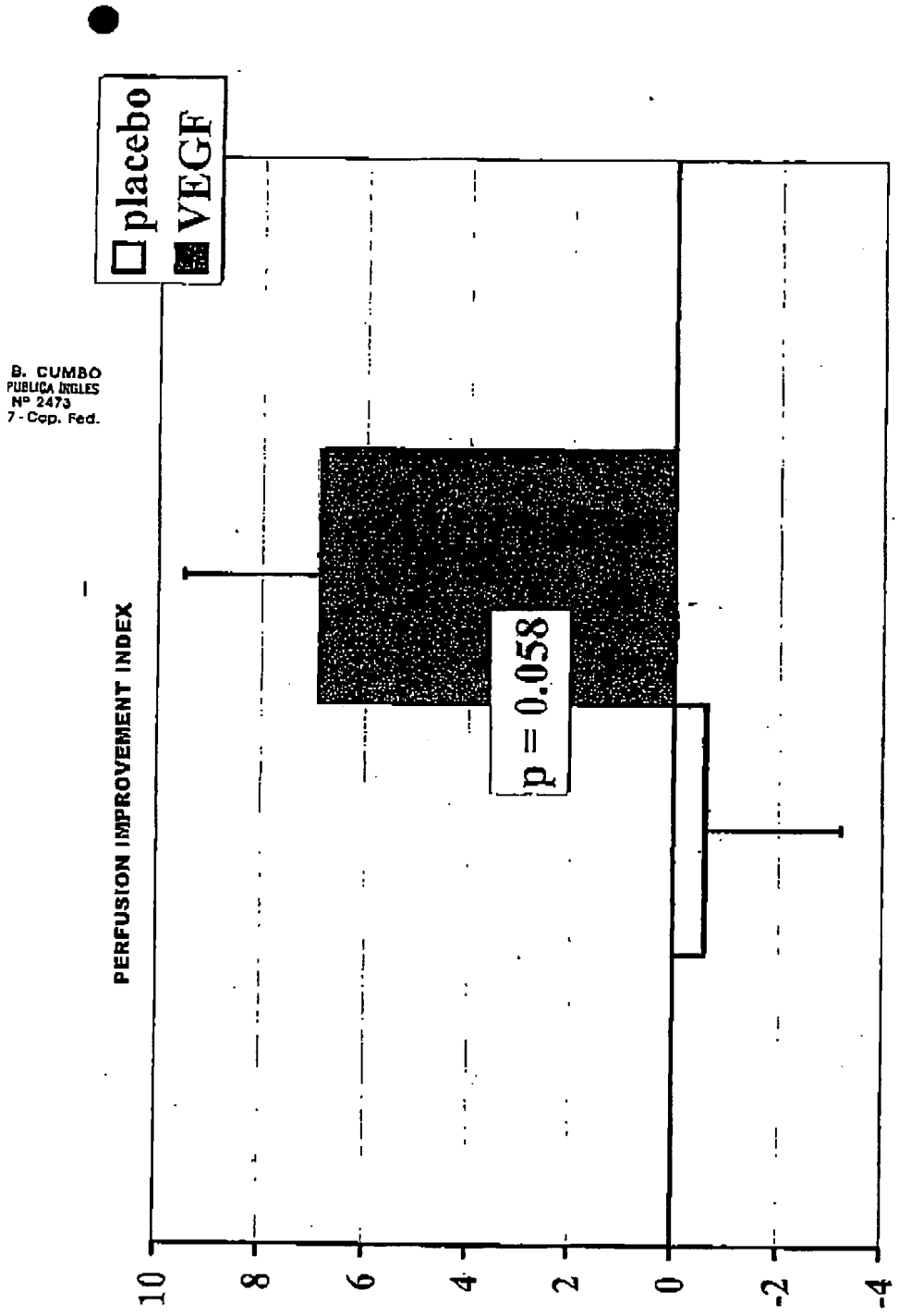
0
-2
-4
-6
placebo
VEGF

Fig. 1


placebo
VEGF
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PERFUSION IMPROVEMENT INDEX
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10
8
6
4
2
0
-2
-4

Fig. 2

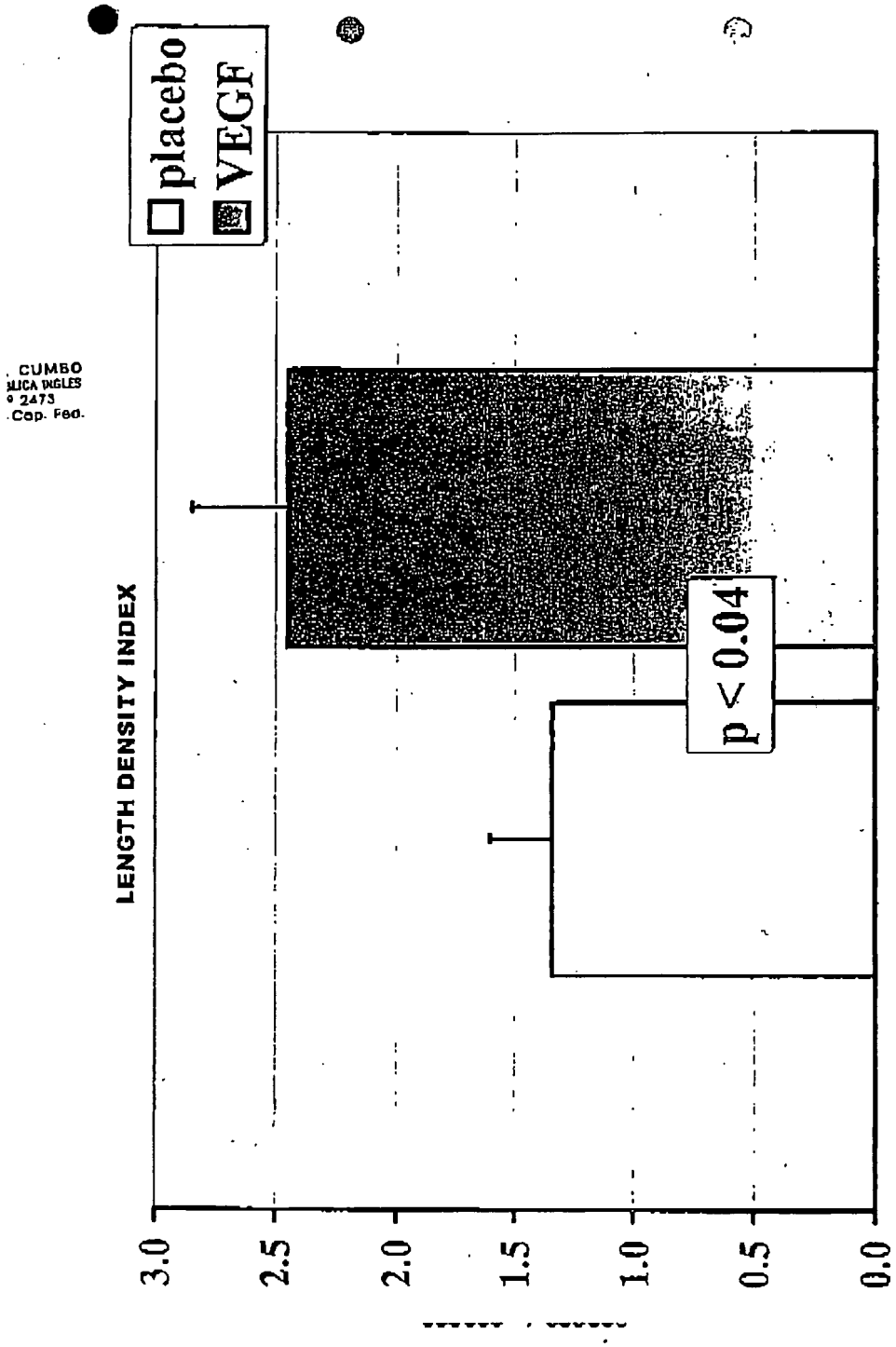


Fig. 3

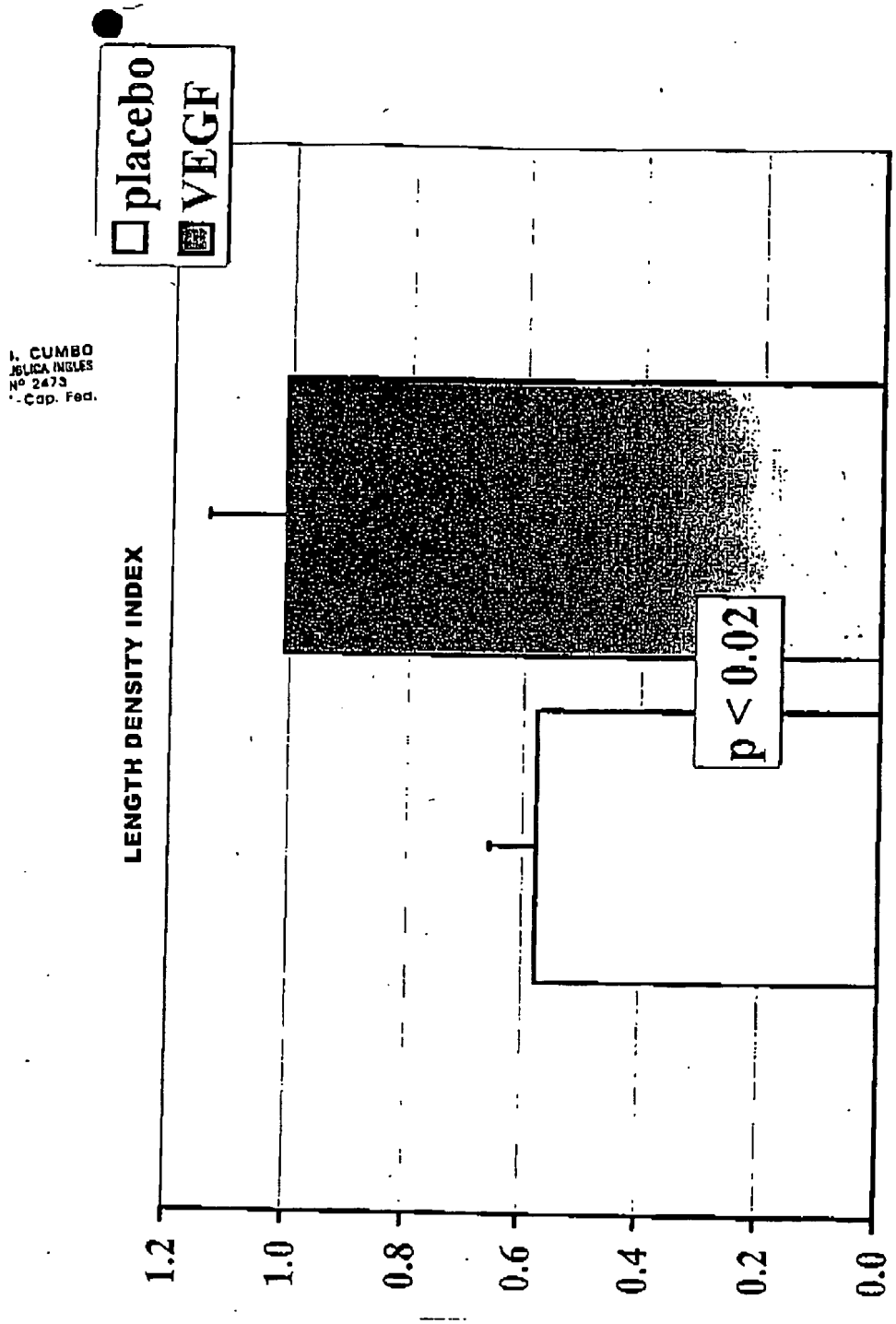
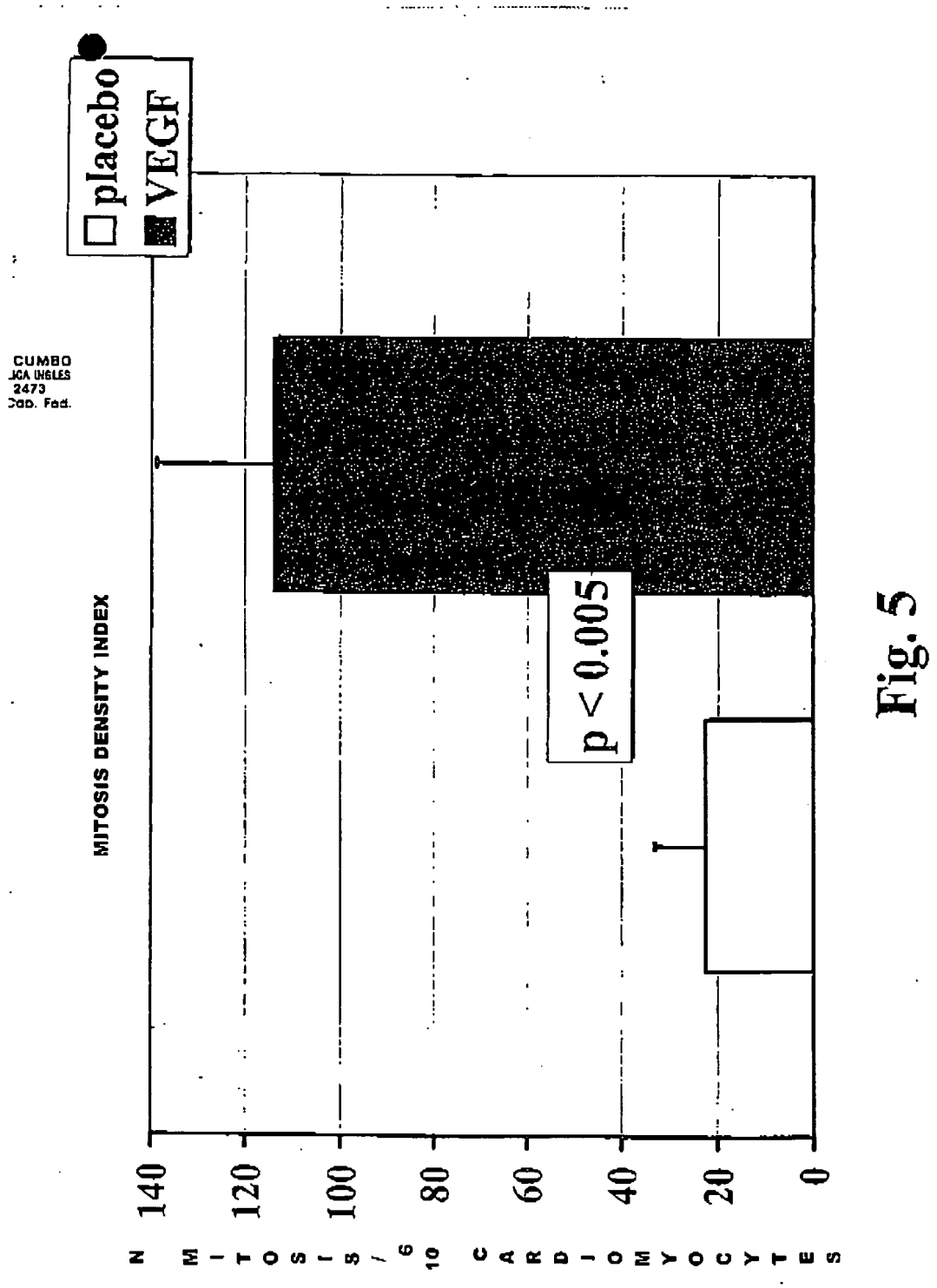


Fig. 4


placebo
VEGF
p < 0.005
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140
120
100
80
60
40
20
0

Fig. 5

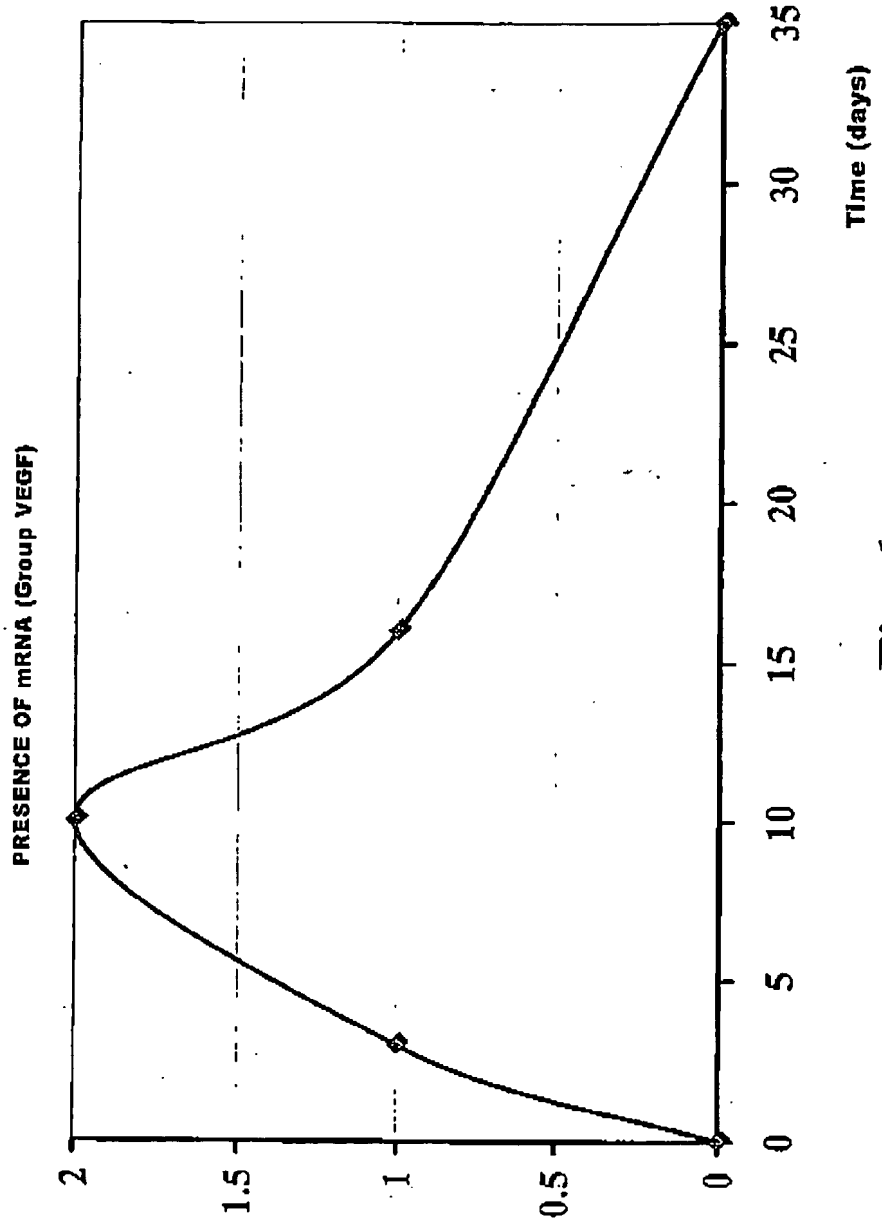


Fig. 6

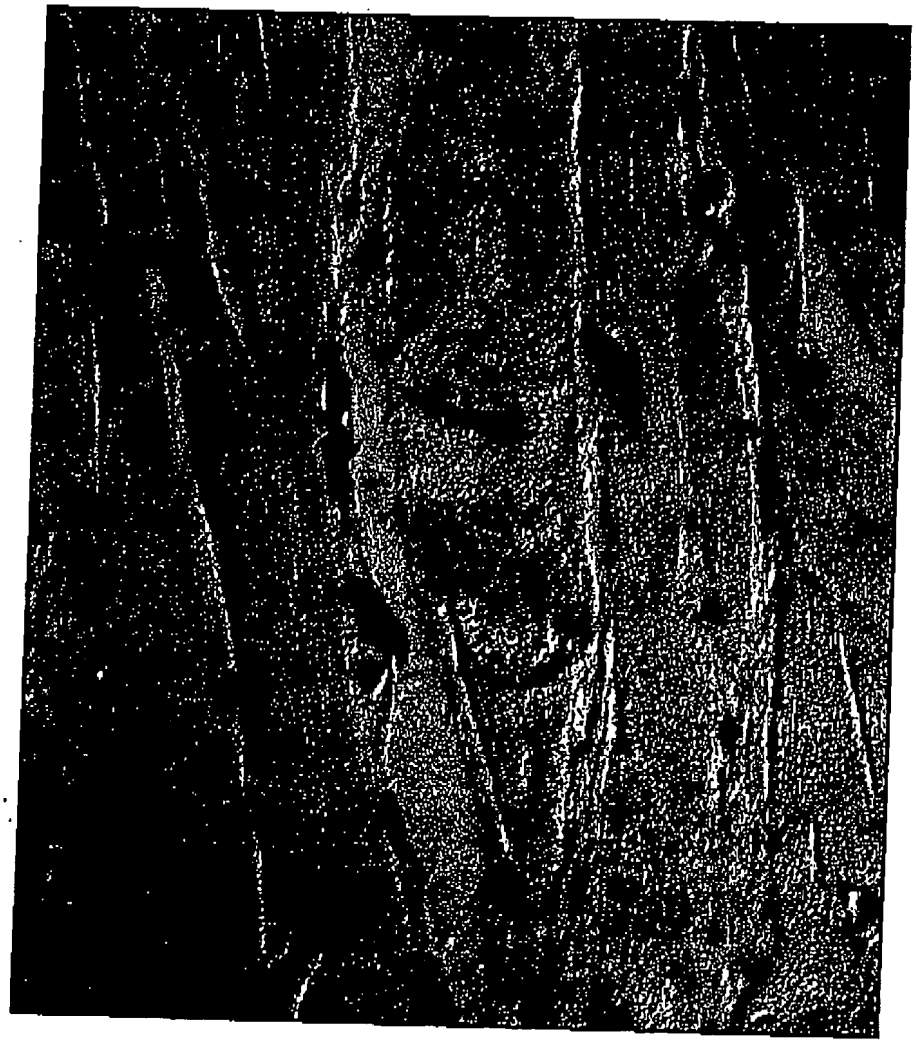


Fig. 7

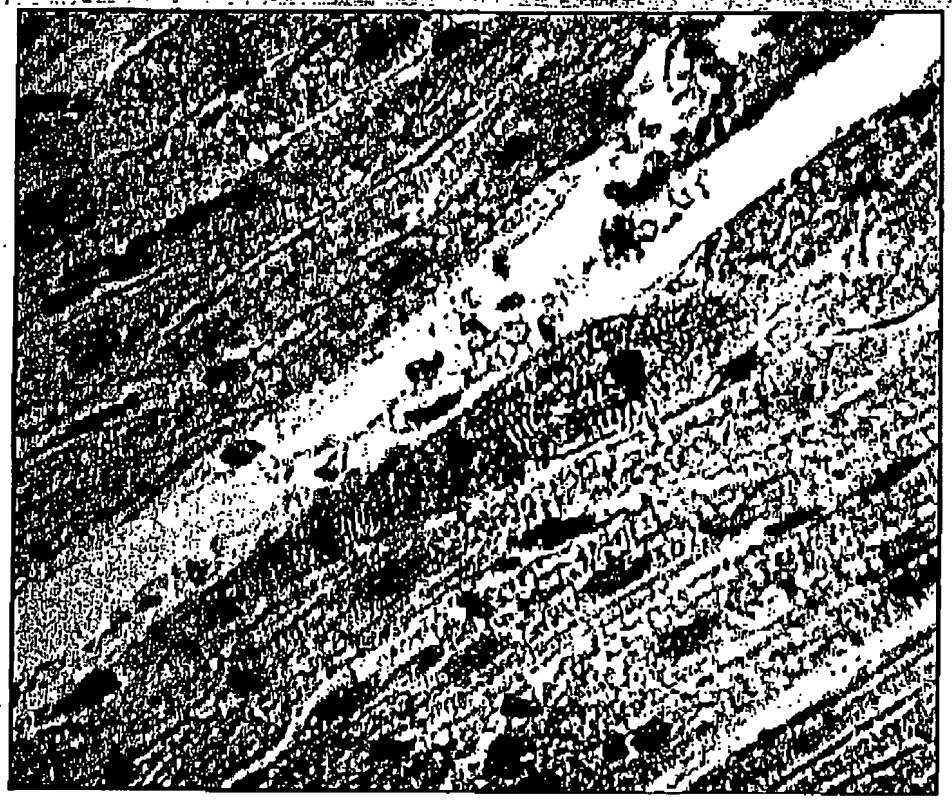
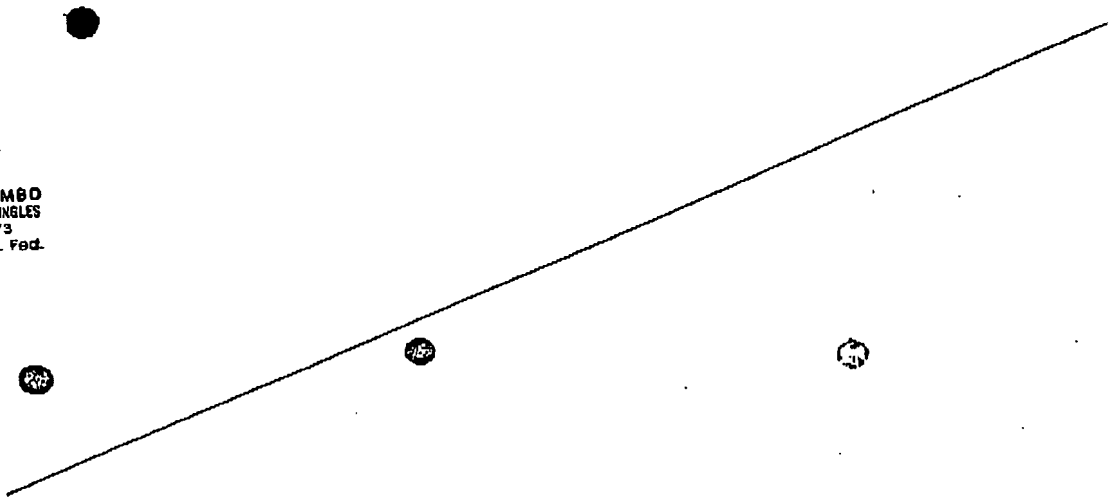


Fig. 8



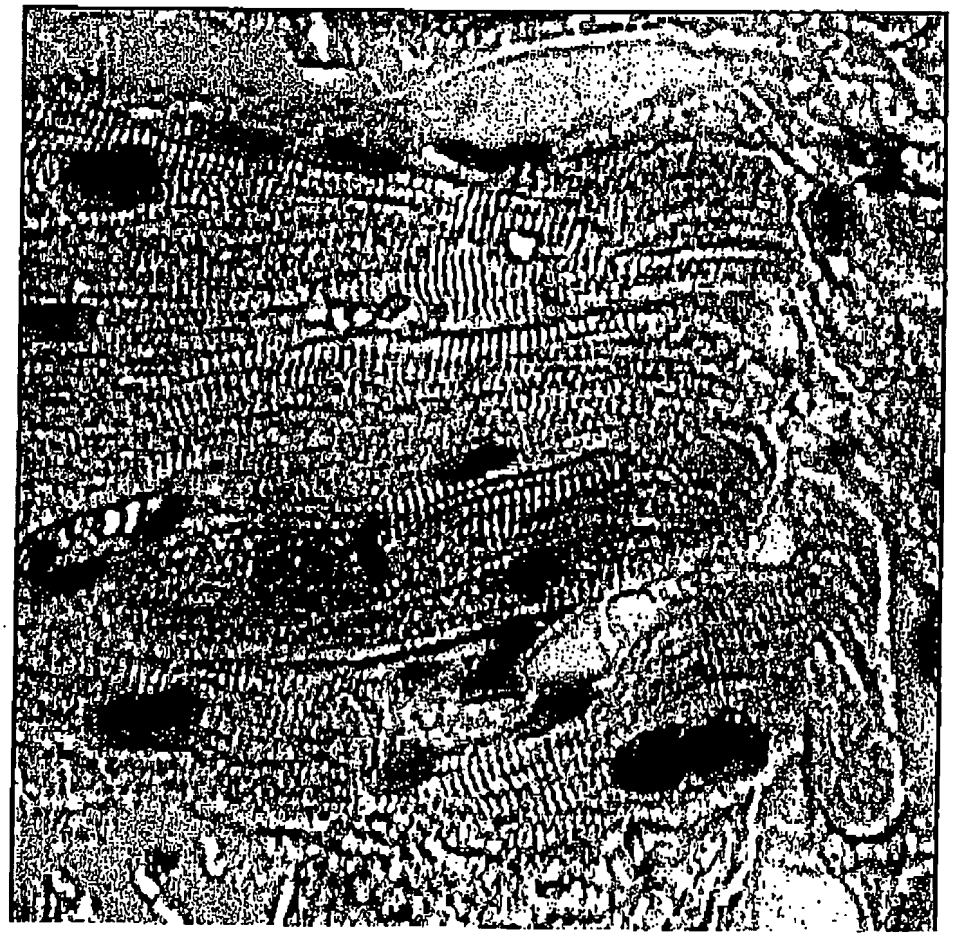


Fig. 9

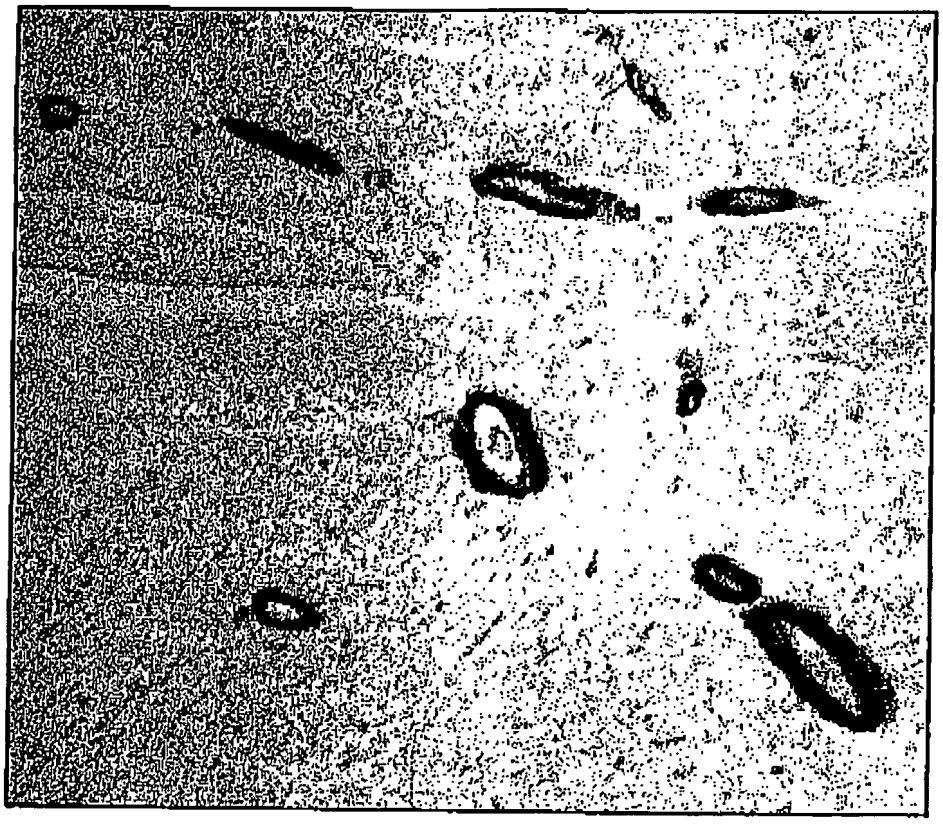


Fig. 10

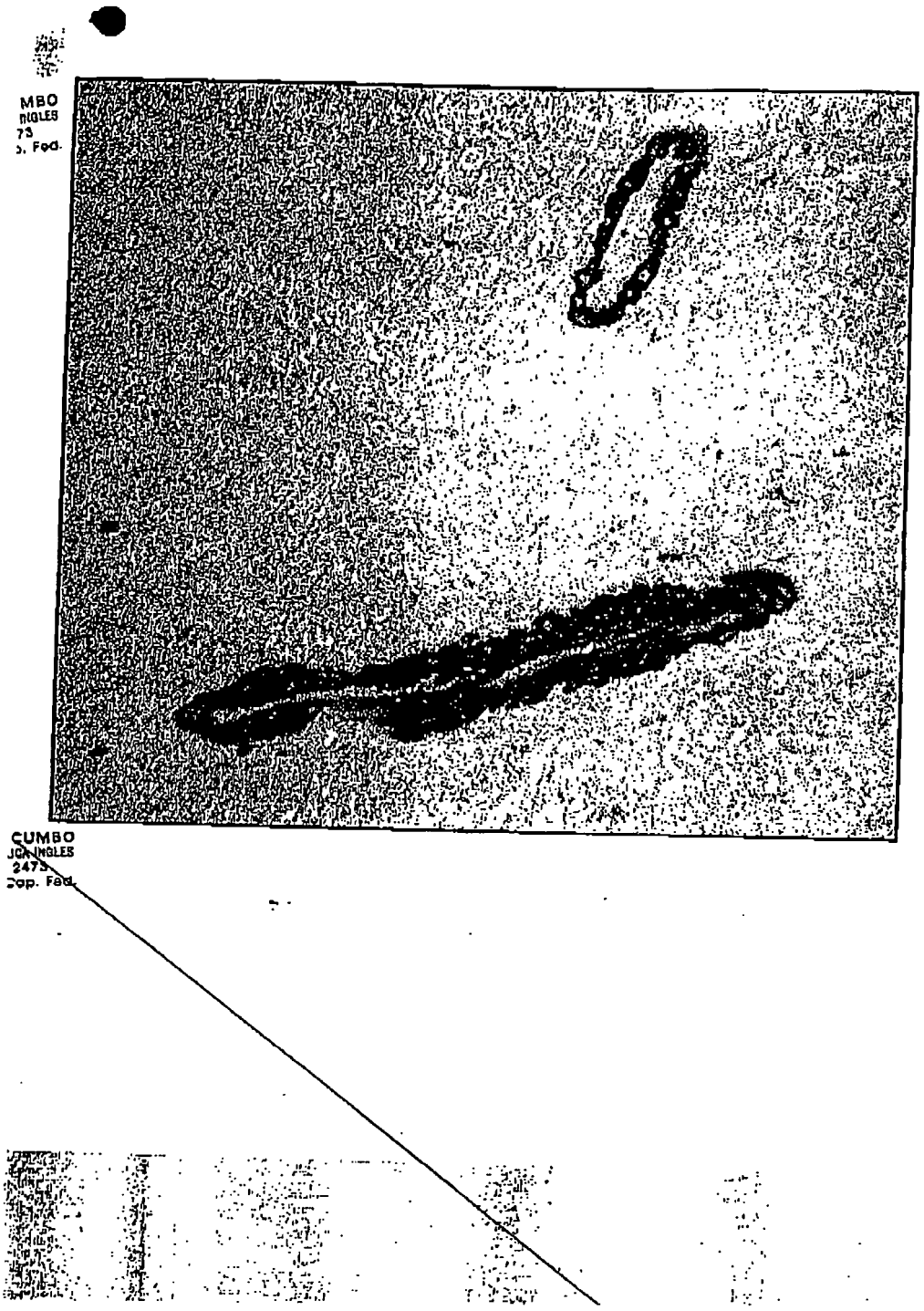


Fig. 11

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TECHNICAL DATASHEET

(10) ——PUBLICATION No.: AR

(21) APPLICATION No.:

On the left, there appears the Argentinian Coat of Arms

(19) I.N.P.I.

Argentine Republic

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(72) Inventor(s):

(74) Agent. 611

(83) Microorganisms deposit

(54) TITLE OF THE INVENTION: "METHOD TO INDUCE NEOVASCULAR PROLIFERATION AND TISSUE REGENERATION".

(57) The present invention refers to a method for inducing neovascular proliferation and tissue regeneration in mammals. The claimed method is characterized for the administration of a nucleotide sequence encoding for the active site of the vascular endothelial growth factor (VEGF), into a tissue. The method induces cell mitosis, myocardiogenesis, and the angiogenesis, arteriogenesis, vasculogenesis, lymphangiogenesis in mammalian tissues. The method employs a plasmid vector for the transport of the encoding nucleotide sequence. The administration is performed by intramyocardial route.

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JBLICA INGLES
Nº 2473
-Cap. Fed.

INVENTION PATENT.----------

Under Proceeding P010102313, an INVENTION PATENT application has been filed----------

Buenos Aires, ----, 2001.----------

RECEPTION DESK.----------

I, VIVIANA BEATRIZ CUMBO, A SWORN PUBLIC TRANSLATOR, DO HEREBY CERTIFY THE FOREGOING TO BE A TRUE TRANSLATION INTO ENGLISH OF THE PHOTOCOPY OF THE ORIGINAL DOCUMENT IN SPANISH LANGUAGE, ATTACHED HERETO, WHICH I HAVE HAD BEFORE ME. DONE AND SIGNED IN BUENOS AIRES, ON THIS NINETEENTH DAY OF JANUARY, TWO THOUSAND AND FOUR.----------

YO, VIVIANA BEATRIZ CUMBO, TRADUCTORA PÚBLICA MATRICULADA, CERTIFICO POR LA PRESENTE, QUE ÉSTA ES TRADUCCIÓN FIEL AL IDIOMA INGLÉS DE LA FOTOCOPIA DEL DOCUMENTO ORIGINAL REDACTADO EN IDIOMA CASTELLANO, ADJUNTA A LA PRESENTE, QUE HE TENIDO A LA VISTA, Y A LA CUAL ME REMITO, EN BUENOS AIRES, A LOS DIECINUEVE DÍAS DEL MES DE ENERO DE DOS MIL CUATRO.----------

COLEGIO DE TRADUCTORES PUBLICOS
DE LA CIUDAD DE BUENOS AIRES
Corresponde a la Legalización
Nº T1-04-6804
FABIANA G. POMILIO

VIVIANA B. CUMBO
TRADUCTORA PUBLICA INGLES
Inscrip. Nº 2473
Tº IX - Fº 347 - Cap. Fed.

N° 00712419

COLEGIO DE TRADUCTORES PÚBLICOS
DE LA CIUDAD DE BUENOS AIRES

REPUBLICA ARGENTINA
LEY 20.305

LEGALIZACIÓN

Por la presente el COLEGIO DE TRADUCTORES PÚBLICOS DE LA CIUDAD DE BUENOS AIRES en virtud de la facultad que le confiere el artículo 10, inc. d) de la Ley 20.305, certifica que la firma y sello que aparecen en el documento adjunto, concuerdan con los correspondientes al traductor CUMBO, VIVIANA BEATRIZ

que obran en nuestros registros en el folio tomo

INGLES 347 9

La presente legalización no juzga sobre el contenido ni sobre la forma del documento.

Buenos Aires, Legalización Número: 1476 / 2004 / T1

Fecha: 28/01/2004

RICARDO ALCOBA LOPEZ
GERENTE GENERAL
COLEGIO DE TRADUCTORES PUBLICOS
DE LA CIUDAD DE BUENOS AIRES

ESTA LEGALIZACIÓN NO ES VÁLIDA SIN EL CORRESPONDIENTE TIMBRADO EN LA ÚLTIMA HOJA DEL DOCUMENTO ADJUNTO

Av. Callao 289 - 4° Piso - 1022 BUENOS AIRES - TEL. 4371-8618 / 4372-7961/ 2961/ 4373-4644